Vaccine 36 (2018) 1528–1532

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Short communication

Comparison of serum bactericidal and antibody titers induced by two *Haemophilus influenzae* type b conjugate vaccines: A phase III randomized double-blind study

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ARTICLE INFO

Article history:
Received 29 November 2017
Received in revised form 1 February 2018
Accepted 3 February 2018
Available online 17 February 2018

Keywords:
Haemophilus influenzae type b
Hib conjugate vaccine
Aluminum phosphate adjuvant
Serum bactericidal activity
Seroprotection threshold

ABSTRACT

Haemophilus influenzae type b (Hib) conjugate vaccines have drastically reduced disease incidence worldwide. Protection against Hib infection has relied on the serum bactericidal activity (SBA) of antibodies to the Hib capsular polysaccharide (polyribosylribitol phosphate). However, licensure usually relies on measuring induction of antibodies to PRP as a surrogate for SBA. In a phase III clinical trial we compared a PRP-conjugate vaccine using the nontoxic diphtheria toxin mutant, CRM_{197}, as carrier protein with the licensed tetanus toxoid conjugate when administered subcutaneously as a three dose primary series in Japanese infants. As an addition to the phase III study, we have now evaluated SBA and show PRP-CRM_{197} induces higher levels of SBA than PRP-T four weeks after the primary series, with a statistically significant correlation with anti-PRP titers. This data confirms the superior immunogenicity of PRP-CRM_{197} compared with PRP-T assessed as SBA following a three-dose primary series by subcutaneous administration.

Clinical trial registry: Registered on ClinicalTrials.gov (NCT01379846).

1. Introduction

The number of pediatric cases of invasive Hib infection in Japan has decreased due to the introduction of a Hib conjugate vaccine (ActHIB®) consisting of PRP conjugated with tetanus toxoid (PRP-T) [1]. We have previously reported on a phase III clinical trial comparing the immunogenicity of Vaxem™ Hib (PRP conjugated with a nontoxic diphtheria toxin mutant, CRM_{197}, containing aluminum phosphate adjuvant [PRP-CRM_{197}]) with PRP-T. Since no significant differences in terms of Hib PRP-specific IgG antibody titers were shown between PRP-CRM_{197} and PRP-T after a primary series of vaccinations in Japanese children, both vaccines were expected to be equally effective in children in Japan [2]. Recognized measures of Hib vaccine-induced protective immunity against Hib infection are anti-PRP antibody concentrations associated with short-term seroprotection (≥0.15 μg/ml) and long-term seroprotection (≥1.0 μg/ml) [3]. The long-term seroprotection rate was reported to correspond to serum bactericidal activity (SBA) of 8 [3]. Several publications have reported that cross-reactive antigens may induce non-functional antibody against Hib [4,5], and vaccine failure was reported to occur in one infant with sufficient anti-PRP antibody (≥1.0 μg/ml) but low SBA titer (SBA = 16) [6]. It has been suggested that SBA is the more reliable predictor of successful immunization against Hib [7–9]. To reinforce the conclusions of our clinical trial mentioned above using PRP antibody titers, we compared SBA titers between PRP-T and PRP-CRM_{197} in samples from the same study.

2. Materials and methods

2.1. Study design

This phase 3, randomized observer-blind multi center, parallel-group study conducted in Japan was previously reported in detail

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https://doi.org/10.1016/j.vaccine.2018.02.011
0264-410X/

[2]. An Institutional Review Board approved the study protocol, which was performed in accordance with the Declaration of Helsinki and Good Clinical Practice Standards and applicable local regulations. Informed consent was obtained from all parents or guardians of the children. The study was registered at ClinicalTrials.gov (NCT01379846).

2.2. Vaccines and vaccination

The Hib vaccines were VAXEM™ Hib, consisting of PRP conjugated with a nontoxic diphtheria toxin mutant, CRM_{197}, containing 0.3 mg of aluminum phosphate adjuvant [Novartis Vaccine and Diagnostics, now a GSK company, and licensed by Takeda Pharmaceutical Company Limited for use in Japan] and ActHIB®, consisting of PRP conjugated to tetanus toxoid (Sanofi Pasteur S.A., Lyon, France). Both vaccines were administered subcutaneously to children aged between 3 and 6 months at the time of the first dose in a three-dose primary series (4 weeks intervals), with a booster vaccination administered at approximately 1 year after completion of the primary series. DTaP vaccine containing 0.1 mg of aluminum hydroxide adjuvant (Adsorbed Diphtheria-Purified Pertussis-Tetanus Combined Vaccine Kit TAKEDA) was administered concomitantly by subcutaneous injection in the contralateral arm.

2.3. Immunogenicity measurement

Four blood samples were drawn; before the first vaccination, and 4 weeks after the third vaccination, and before and 4 weeks after the booster vaccination. Anti-PRP IgG was measured by using VaccZyme™ Human Anti-*Haemophilus influenzae* type b Enzyme Immunoassay Kit (The Binding Site Group, UK), according to the manufacturer's instructions. In this additional analysis, SBA was measured in the samples taken before and after the primary series at Shin Nippon Biomedical Laboratories, Ltd following a previously reported method with minor modifications [8]. Briefly, *H. influenzae* GB3291 was grown overnight in brain heart infusion (BHI) medium supplemented with NAD (nicotinamide adenine dinucleotide) and hemin at final concentrations of 10 μg/ml each, then stored at −80 °C until use. Heat-inactivated serum samples were added to wells of microtiter plates and serially diluted twofold starting at 1:8 in dilution buffer (Hanks buffer with Ca^{2+} and Mg^{2+}, supplemented with 2% BHI medium, 10 μg/ml NAD and 10 μg/ml hemin). *H. influenzae* (10^3 bacteria/well), followed by 25 μl of rabbit complement (Pel-Freez) and 25 μl of alamar Blue solution (16% alamar Blue [Trek Diagnostics], 64% dilution buffer, and 2% BHI medium), were added to each of the microtiter plate wells before incubation at 37 °C for 6 h in a 5% CO_2 atmosphere and then fluorescence measurement (Ex/Em = 530/590 nm). SBA titers were expressed as the reciprocal of the serum dilution that resulted in 50% killing of the initial inoculum compared with that achieved with the fluorescence of a known concentration (in colony-forming units, CFU) of bacterial controls. An SBA titer less than the detection limit (<16) was considered to be 16, and only correlations between SBA titer ≥16 and anti-PRP antibody concentration were evaluated.

2.4. Statistical analysis

The study was designed to test the non-inferiority of PRP-CRM_{197} vaccine to PRP-T vaccine in terms of anti-PRP antibody response measured by seroprotection rate (titer ≥1.0 μg/mL) at 4 weeks after the third dose in the primary series (primary endpoint) [2]. The SBA titer was set as an exploratory endpoint. With regard to the anti-PRP antibody response and SBA, the geometric mean titer (GMT) and two-sided 95% confidence interval (95% CI) were calculated for each group, and the geometric mean ratio (GMR, PRP-CRM_{197}/PRP-T) and 95% CI were also calculated. In addition, the correlation between anti-PRP IgG antibody concentration and SBA titers above the lower limit (16) was analyzed for all subjects in an exploratory analysis that was not prespecified in the statistical analysis plan. Statistical analyses were performed using SAS Ver.9.2 (SAS Institute Inc.).

3. Results

A total of 415 children were immunized with three doses of Hib vaccine (either PRP-CRM_{197} or PRP-T) given as a primary series and a booster vaccination [2]. As described previously [4], the level of anti-PRP specific IgG was increased after the primary series with PRP-CRM_{197} (18.0 μg/ml), or PRP-T (8.67 μg/ml). Proportions of subjects with titers above the threshold for long-term seroprotection were 99.3% and 95.6% in the PRP-CRM_{197} and PRP-T groups, respectively. Using the same sera we found a significantly higher SBA titer in the PRP-CRM_{197} group than in the PRP-T group (GMR, 2.88 (PRP-CRM_{197}/PRP-T)). Post-immunization geometric mean SBA titers were 201.7 for PRP-CRM_{197}, and 69.9 for PRP-T (Table 1). This difference is evident as shown in reverse cumulative distribution plot for SBA titer (Fig. 1.A). Although there was a moderate correlation between anti-PRP antibody levels and SBA titers for PRP-CRM_{197} (n = 263, r = 0.3543, p < 0.0001) and PRP-T (n = 115, r = 0.3349, p = 0.0003) (Fig. 1.B), a few subjects with ≥1.0 μg/ml (long-term) in both groups who had an SBA titer <16. These subjects may explain the cases of vaccine failure or breakthrough infection of Hib that have been described historically to occur following vaccination. Consequently, subjects with anti-PRP IgG antibody titers ≥0.15 μg/ml (short-term) or ≥1.0 μg/ml (long-term) were categorized as non-responders (SBA < 16) or responders (SBA ≥ 16), and their numbers were compared in each group. This analysis indicated that immunization with PRP-CRM_{197} resulted in significantly fewer non-responders than those who received PRP-T

Table 1
PRP-CRM_{197} and PRP-T immunogenicity as assessed in phase III clinical trial.

Assays	PRP-CRM_{197}		PRP-T
Pre-immunization			
Anti-PRP IgG (μg/ml), (95%CI, n)	0.181 (0.1634–0.1998, 278)		0.171 (0.1502–0.1951, 137)
SBA titer (95%CI, n)	16.18 (15.908–16.467, 272)		16.05 (15.954–16.142, 130)
Post-immunization			
Anti-PRP IgG (μg/ml), (95%CI, n)	18.001 (16.0307–20.2145, 275)		8.674 (7.1871–10.4683, 135)
% Seroprotection (≥1.0 μg/ml), (95%CI)	99.3 (97.398–99.912)		95.6 (90.577–98.352)
GMR (PRP-CRM_{197}/PRP-T)		2.08* (1.6809–2.5624)	
SBA titer (95%CI, n)	201.69 (177.881–228.694, 270)		69.92 (58.679–83.310, 132)
GMR (PRP-CRM_{197}/PRP-T)		2.88* (2.3223–3.5832)	

SBA, serum bactericidal activity; GMR, geometric mean ratio; *, significant (lower limit of 95% CI of GMR exceeds 1).
The anti-PRP IgG data were previously reported [2].